Archives of Insect Biochemistry and Physiology 31:237–256 (1996)

Sex-Specific and Hormone-Controlled Expression of a Vitellogenin-Encoding Gene in the Gypsy Moth

John J. Adamczyk, Jr., Howard W. Fescemyer, David G. Heckel, Linda J. Gahan, Robin E. Davis, and Thomas J. Kelly

Departments of Entomology (J.J.A., H.W.F.) and Biological Sciences (D.G.H., L.J.G.), Clemson University, Clemson, South Carolina; Insect Neurobiology and Hormone Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Beltsville, Maryland (R.E.D., T.J.K.)

Microvitellogenin and vitellogenin cDNA from *Manduca sexta* (tobacco hornworm) were tested for use as molecular probes to investigate the expression of genes coding for vitellogenins in *Spodoptera frugiperda* (fall armyworm) and *Lymantria dispar* (gypsy moth). Cross-hybridization was not observed between the *M. sexta* cDNAs and *S. frugiperda* DNA and mRNA. Vitellogenin cDNA from *M. sexta* did not hybridize to *L. dispar* DNA or mRNA. However, the 834 bp microvitellogenin cDNA from *M. sexta* hybridized to an approximately 850 bp transcript in *L. dispar* mRNA.

A 2.5 kb cDNA clone, pz64, was isolated from late last instar larvae of female *L. dispar* by differential screening. This clone has 38% amino acid sequence (deduced) and 55% nucleic acid sequence similarities with the 3′-end of high molecular weight vitellogenin in *Bombyx mori* (silkworm). When used as a probe in northern analysis of *L. dispar* mRNA, this cDNA hybridized to a 5.3 kb transcript in female last instar larvae, pupae, and adults, but not to male last instar larvae and adults. This cDNA did not hybridize to mRNA from *M. sexta* or *S. frugiperda*. Expression of the 5.3 kb vitellogenin transcript hybridizing to the 2.5 kb cDNA clone was suppressed in 5-day-old last instar larvae of

Acknowledgments: The authors thank Drs. S. Randal Voss, Laura L. Georgi, John H. Law, and Jay Y. Bradfield for their technical advice and DNA probes. We are grateful for the assistance of Dannie D. Cannon, David A. Jenkins, Daniel R. Lewis, Meredith Mitchell, and M. Ian Smathers. Special thanks go to the United States Biochemical Corporation for assisting in nucleic acid sequencing and the donation of molecular biology supplies. Special recognition and thanks goes to Charlie E. Rogers for the service, advice, and support he provided. Financial support was provided by HATCH project 66-1437 allocated to the South Carolina Agricultural Experiment Station (SCAES), Research Support Agreements (T2-6602-011, T3-6602-011, and T4-6602-011) with the USDA ARS, Tifton, GA, the USDA CSREES Research Apprenticeship Program for Minority Students (94-COOP-2-0255), the SCUREF Summer Scholars Program (SCUREF/U.S. Department of Energy Cooperative Agreement DE-FC09-93SR18262), and a National Science Foundation EPSCoR grant to the State of South Carolina. This paper is technical contribution 4083 of the SCAES, Clemson University.

Received April 17, 1995; accepted September 8, 1995.

Address reprint requests to Howard W. Fescemyer, Clemson University, Department of Entomology, 114 Long Hall, Box 340365, Clemson, SC 29634-0365.

female *L. dispar* treated on day 2 with doses of the juvenile hormone analog, methoprene, greater than 10 nmol. Apparently, the high in vivo titer of juvenile hormone during the first 2 days of the last instar represses the transcription of vitellogenin mRNA.

Key words: development, hormonal control, insect, Lepidoptera, molecular biology, reproduction, sequence homology

INTRODUCTION

Vitellogenins are a unique group of proteins that are precursors of the major egg yolk proteins, vitellins (Hagedorn and Kunkel, 1979). Except for a few species, mainly the higher dipterans, nearly all insect vitellogenins are synthesized in the fat body before the onset of yolk deposition (Kelly et al., 1992). Vitellogenins are transported via the hemolymph to the follicular epithelium (Engelmann, 1983) which incorporates them into the yolk of the developing oocyte. These female-specific proteins are involved in the maturation of eggs in insects and eventually make up 80–90% of the total protein content of mature eggs. Vitellogenins are present in all female Lepidoptera studied. They have a native molecular weight of 400–500 kDa and consist of 2–3 apoprotein subunits depending on the species (Kunkel and Nordin, 1985; Shirk, 1987; Davis et al., 1990a; Satyanarayana et al., 1992; Cusson et al., 1994).

Microvitellogenin is another type of yolk protein found in the giant silkmoth, *Hyalophora cecropia* (L.) (Lepidoptera: Saturniidae), and the tobacco hornworm, *Manduca sexta* (L.) (Lepidoptera: Sphingidae) (Kawooya and Law, 1983), where its size is approximately 26 kDa (Wang et al., 1988). Like vitellogenin, microvitellogenin is female-specific, expressed in fat body, and sequestered from the hemolymph by the follicular epithelium (Kawooya and Law, 1983; Kulakosky and Telfer, 1989; Wang et al., 1988, 1989). Expression of microvitellogenin appears to be regulated by 20-hydroxyecdysone (Wang et al., 1989).

The timing of vitellogenin expression during development is species-specific and regulated by juvenile hormone, ecdysteroids, or both (Kelly et al., 1992). Juvenile hormone and its analogs, such as fenoxycarb and methoprene, stimulate vitellogenin expression which occurs only in the adults of many Lepidoptera. Examples of species with this regulatory pattern are the monarch butterfly, *Danaus plexippus* (L.) (Danaidae) (Pan and Wyatt, 1976), and the noctuid moths such as the corn earworm, *Helicoverpa zea* (Boddie) (Satyanarayana et al., 1992), the true armyworm, *Pseudaletia unipuncta* (Haworth) (Cusson et al., 1994), and the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Fescemyer, unpublished data).

On the other hand, vitellogenin expression in some Lepidoptera is associated with the endocrine events regulating metamorphosis of the larva, pupa, and pharate adult. Juvenile hormone and ecdysteroids regulate the expression of vitellogenin, but the role of these hormones depends on the stage of development. Examples of species with this multiple regulatory pattern are *M. sexta* (Satyanarayana et al., 1994), the Indianmeal moth, *Plodia interpunctella* (Hübner) (Pyralidae) (Shirk et al., 1992), the silkworm, *Bombyx mori* (L.) (Bombycidae) (Yano et al., 1994), and the gypsy moth, *Lymantria dispar* (L.) (Lymantriidae) (Davis et al., 1990b; Fescemyer et al., 1992; Hiremath and Jones, 1992).

Vitellogenin synthesis and accumulation in the hemolymph first occurs early (days 2–3) in last instar larvae of female *L. dispar* (Davis et al., 1990a; Lamison et al., 1991; Fescemyer et al., 1992). In addition, vitellogenin expression is suppressed rather than stimulated by juvenile hormone or its analogs (Davis et al., 1990b; Fescemyer et al., 1992; Hiremath and Jones, 1992; Hiremath et al., 1994). During the first 3 days of last instar larvae, the decline in juvenile hormone level associated with larval-pupal metamorphosis (Tanaka et al., 1989) is necessary for the initiation of vitellogenin expression and its accumulation in the hemolymph of female *L. dispar* larvae (Fescemyer et al., 1992; Hiremath and Jones, 1992; Hiremath et al., 1994). Information about the gene coding for vitellogenin is necessary for elucidating the regulatory regions of the gene and the mechanism by which juvenile hormone represses vitellogenin expression in *L. dispar.*

Comparisons among the available nucleotide and protein sequences suggest that there are three gene families for proteins that accumulate in the yolk of animal eggs. One family consists of the high molecular weight vitellogenins found in nematodes, vertebrates, sea urchins, and some insects (Blumenthal and Zucker-Aprison, 1987; Nardelli et al., 1987) such as locusts (Blumenthal and Zucker-Aprison, 1987) and the boll weevil, *Anthonomus grandis* (Boheman) (Coleoptera: Curculionidae) (Trewitt et al., 1992). A second gene family consists of the low molecular weight vitellogenins and yolk proteins of the higher Diptera, such as *Drosophila melanogaster* (Meigen) (Drosophilidae) and the mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Tephritidae) (Blumenthal and Zucker-Aprison, 1987; Rina and Savakis, 1991). The third family consists of microvitellogenin from *M. sexta* (Wang et al., 1988).

Little is known about the genes coding for vitellogenin in Lepidoptera. Yano et al. (1994) recently reported the 5.4 kb nucleotide sequence for vitellogenin mRNA in *B. mori.* Hiremath and Jones (1992) reported on the presence of an approximately 5.4 kb poly(A)$^+$ RNA in last instar larvae of female *L. dispar.* Synthesis of this 5.4 kb RNA and its translation into the 185 kDa vitellogenin subunit was suppressed by the juvenile hormone analog fenoxycarb. Hiremath et al. (1994) reported the nucleotide sequence of an 822 bp cDNA representing an internal region of this 5.4 kb RNA which appears to be the mRNA for *L. dispar* vitellogenin.

The present study was initiated to identify vitellogenin genes and transcripts in *S. frugiperda* and *L. dispar* for investigation of the hormonal regulation and developmental timing of their expression. The cDNAs for microvitellogenin and vitellogenin in *M. sexta* were used as molecular probes to identify and compare vitellogenin genes and transcripts among these moths. Also, a 2.5 kb female-specific cDNA clone coding for the 3′-end of the *L. dispar* vitellogenin gene was prepared from the fat body of female *L. dispar.* This cDNA was used as a molecular probe to investigate suppression of vitellogenin transcription by juvenile hormone, and to document the developmental timing of vitellogenin transcription in larval, pupal, and adult stages of female *L. dispar.*

MATERIALS AND METHODS

Insect Rearing and Hormone Treatment

M. sexta pupae were obtained from R. M. Roe, Department of Entomology, North Carolina State University, Raleigh, NC. These pupae were held at 27°C until adult eclosion. Adults were quickly frozen in liquid nitrogen on the day of eclosion. New Jersey strain *L. dispar* of various larval developmental stages were obtained from T. J. Kelly. These insects were reared, staged, and sexed as described in Fescemyer et al. (1992). All *L. dispar* were quickly frozen in liquid nitrogen, shipped on dry ice to H. W. Fescemyer in 24 h, and stored at –80°C.

Dosages of the juvenile hormone analog, methoprene (ChemService, Inc., Westchester, PA) were prepared in ethanol. These dosages were topically applied (2 µl) to the dorsum of the abdomen of 2-day-old last (5th) instar larvae of female *L. dispar*. All treated larvae were frozen on day 5 as described above.

Spodoptera frugiperda were from a laboratory colony established in September 1992, with a mixture of progeny from single pair matings of adults from larvae collected from peanut near the Edisto Research and Education Center, Blackville, SC. Analyses of allozyme differences (Pashley, 1986) and restriction fragment length polymorphism for mitochondrial DNA (Pashley, 1989) were used to determine that a hybrid colony was probably established (Heckel et al., manuscript in preparation). Larvae and adults were reared according to methods described in Perkins (1979) as modified by the use of a soybean/wheat germ meridic diet (King and Hartley, 1985). All stages were held at 27 ± 1°C, relative humidity of 70–80%, and 14:10 h (light:dark) photoperiod.

Isolation of Genomic DNA

A modification of the method developed by Kirby (1957) was used to homogenize and extract genomic DNA from approximately 50 mg of frozen (–80°C) tissue, such as the head and thorax of an adult *S. frugiperda*. The tissue was homogenized in buffer (0.2 M NaCl, 50 mM Tris-HCl, pH 8.0, 1.0 mM DTT, 10 mM EDTA, 0.2% SDS). Phenol and chloroform were used to extract DNA from the homogenate. The DNA in the aqueous phase was recovered by precipitation in cold (–20°C for approximately 45 min) ethanol and centrifugation (12,000*g* for 5 min). This DNA was dried in a vacuum desiccator for 10–20 min, suspended in 100 µl of TE buffer, and stored at –20°C.

Isolation of Total RNA and Poly(A)$^+$RNA

Extreme care was used at all times to ensure sterile conditions. Ribonuclease activity on glassware and plasticware was reduced by autoclaving. All solutions were made with HPLC water which had been treated with 0.1% diethylpyrocarbonate and then autoclaved. Fat body tissue was dissected as described in Fescemyer et al. (1992). The method of Chomczynski and Sacchi (1987) as modified by Puissant and Houdebine (1990) was used to obtain total RNA from whole abdomen tissue or about 1 g of fat body tissue. Poly(A)$^+$ RNA was isolated from total RNA with the mini-oligo(dT) cellulose spin column protocol of 5 Prime-3 Prime, Inc., Boulder, CO. The poly(A)$^+$RNA-containing eluant from the column was combined with 20 µl of 1 mg/ml mussel glycogen, 100 µl of 3 M sodium acetate (pH 5.2), and 2.5 volumes of cold ethanol before it was stored at –80°C to precipitate the RNA.

Gel Electrophoresis of DNA and RNA

Agarose gel electrophoresis (Voytas, 1988) run horizontally at constant voltage of 1–5 V/cm was used to resolve DNA. Gels typically contained 0.8–1.5% of high-melting point agarose. Gels were stained in ethidium bromide for 10 min and destained in water for 25–30 min. The DNA in the gels was visualized with an ultraviolet light box at 312 nm and photographed. Band sizes of DNA were estimated by regression analysis of the mobility and fragment sizes of HindIII digested λDNA. Poly(A)$^+$RNA was resolved with agarose/formaldehyde (denaturing) gel electrophoresis (Lehrach et al., 1977) run horizontally at constant voltage of 5 V/cm. Band sizes of RNA were estimated by regression analysis of the mobility and fragment sizes of a 0.24–9.46 kb synthetic RNA ladder generated from λDNA (Life Technologies, Gaithersburg, MD).

cDNA Synthesis, Cloning, and Screening

Double-stranded cDNA was synthesized with a cDNA kit (Pharmacia Biotech Inc., Piscataway, NJ) using about 5 μg of poly(A)$^+$RNA from fat body dissected out of 6- and 7-day-old last instar larvae of female *L. dispar*. Briefly, the RNA was reverse transcribed using oligo $d(T)_{12\text{-}18}$ as a primer. Estimated mass conversion rate for first-strand synthesis with [^{32}P]dCTP was 27%. Gel electrophoresis and autoradiography of the [^{32}P]-labeled reaction products showed major bands of about 0.5, 1.3, 2.0–2.3, and 5.5 kb (data not shown) which would be of sufficient length to encode for polypeptides of about 18.5, 48, 74, 85, and 204 kDa. Double-stranded cDNA was blunt-ended with the Klenow fragment and ligated to EcoR I linker/adapters. The cDNA was purified by extraction with equal parts of phenol and chloroform/isoamyl alcohol (24:1) followed by Sephacryl® S-200 gel spin-column chromatography. This cDNA was used to construct a library in the expression vector lambda ZAP® II (Stratagene, La Jolla, CA). The vector was digested with EcoR I, treated with calf intestine alkaline phosphatase, and 0.5 μg was ligated to 270 ng of cDNA and packaged in vitro with Gigapack II packaging lysates (Stratagene) to yield 3×10^5 plaque forming units.

Approximately 55,000 unamplified clones in the library were tested for female-specific clones by plus/minus screening. Probes used were high specific activity [^{32}P]dCTP-labeled cDNA (Gerard, 1988) synthesized from poly(A)$^+$RNA isolated from last instar larvae of male (pooled days 1, 5, 8, and 9) or female (pooled days 6 and 7) *L. dispar*. Duplicate nitrocellulose plaque lifts (Benton and Davis, 1977) were obtained from the same plate. One of the plaque lifts was probed (Benton and Davis, 1977; Huynh et al., 1985) with male [^{32}P]cDNA while the other plaque lift was probed with female [^{32}P]cDNA. The autoradiographs of these plaque lifts were compared for clone selection. Seventy clones with little or no hybridization to male [^{32}P]cDNA but strong hybridization to female [^{32}P]cDNA were selected. These were plaque-purified and replated at a low plaque density to ensure good separation on the plate. Duplicate nitrocellulose plaque lifts were obtained from these plates and probed with male and female [^{32}P]cDNA as described above. This second screening resulted in seven clones being identified as female-specific. These clones were plaque-purified, and the pBluescript phagemid and ac-

companying insert were excised. Plasmid miniprep DNA (Qiagen Plasmid Kit, Qiagen, Chatsworth, CA) was digested with EcoR I and electrophoresed on 1.2% agarose as described above. These clones had DNA inserts ranging in size from 260–2,700 bp (Kelly et al., 1992).

Additional Probes

In addition to the seven *L. dispar* cDNA clones isolated as described above, the following two cDNA clones from *M. sexta* were also used as probes. A plasmid (pTZ18U) containing the 834 bp insert of microvitellogenin cDNA from *M. sexta* (Wang et al., 1988, 1989) was a gift from J. H. Law, Department of Biochemistry, University of Arizona, Tucson. A plasmid (pBluescript SK^+) containing the 2.1 kb insert of vitellogenin cDNA from *M. sexta* (Satyanarayana et al., 1994) was a gift from J. Y. Bradfield, Department of Entomology, Texas A&M University, College Station.

All plasmids were transferred into *Escherichia coli* (JM83) to obtain a permanent working stock of clones and to amplify the number of cDNA copies. The plasmids were isolated from the bacteria by using the QIAprep-spin Plasmid Kit, and were electrophoresed on a 1% agarose gel to examine the quality of the plasmid isolates. The cDNA inserts were isolated from the plasmids by digesting the plasmids with either EcoR I or BamH I, separating the plasmid digests on a 1% agarose electrophoresis gel, and extracting the cDNA from the agarose by the method of Tautz and Renz (1983). A random primed DNA labeling kit (United States Biochemical Corp., Cleveland, OH) was used to radiolabel cDNA probes.

Southern Analysis

Restriction digested DNA (about 10 μg) was separated by agarose gel electrophoresis (described above) for 12–16 h at constant voltage of 1–2V/cm. After electrophoresis the gel was washed with 0.25 M HCl for 30 min and then rinsed with HPLC water. An upward capillary transfer to a positively charged nylon membrane (Hybond™-N^+, Amersham Corp., Arlington Heights, IL) was made in 0.4 M NaOH (Southern, 1975; Chomczynski, 1992). Blots were sealed in air-tight bags and stored at 4°C.

Hybridizations were conducted in heat-sealable bags containing 0.2 ml/cm^2 of phosphate hybridization buffer preheated to 55°C. The blot was incubated for 2 h at 55°C prior to introducing the denatured (100°C for 10 min) radiolabeled cDNA probe. The blot was incubated with the probe for 12–16 h at 55°C, after which the membrane was washed in phosphate buffer. Moderate stringency conditions consisted of two washes of the membrane with sodium phosphate buffer at 42°C. High stringency conditions consisted of an additional wash with sodium phosphate buffer at 65°C. The washed blot was wrapped in cling film, and autoradiography was performed according to Ausubel et al. (1988).

Northern Analysis

Poly(A)$^+$RNA (about 10 μg) was separated by denaturing gel electrophoresis as described above. After electrophoresis the gel was rinsed three times with HPLC water. An upward capillary transfer to a Hybond-N membrane

was made in 20 × SSPE buffer. The RNA was fixed onto the membrane by baking at 80°C for 2 h. The blot was sealed in air-tight bags and stored at 4°C. The quality of a Northern blot was monitored by staining it for 45 s with 0.03% methylene blue in 0.3 M sodium acetate (pH 5.2) followed by destaining for 2 min in distilled water. A uniform streak containing some distinct bands indicated that the blot was of high quality.

Hybridizations were conducted in heat-sealable bags containing 15 ml of hybridization buffer and sheared herring sperm DNA (0.3 mg) that was denatured by heating (100°C for 5 min). The blot was incubated at 42°C for 2 h prior to introducing the denatured (100°C for 10 min) radiolabeled probe. The blot was incubated with the probe for 12–16 h at 42°C, after which the membrane was washed in a series of decreasing salt solutions containing 0.1% SDS. Low stringency conditions consisted of two washes of the membrane with 2 × SSPE containing 0.1% SDS and a wash with 1 × SSPE containing 0.1% SDS. Moderate stringency conditions consisted of an additional wash with 0.2 × SSPE containing 0.1% SDS. The washed blot was wrapped in cling film, and autoradiography was performed according to Ausubel et al. (1988).

Nucleic Acid Sequencing

The plasmid (1.5 μg) containing the 2.5 kb insert of cDNA clone pz64 from *L. dispar* was purified using the Qiagen Plasmid Kit. This plasmid was prepared for sequencing by using the Taq DyeDeoxy™ Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA). Polymerase chain reaction (PCR) conditions were a 96°C hot start followed by 25 cycles of the following series: 96°C for 30 s, 50°C for 15 s, 60°C for 4 min. Oligodeoxynucleotide primers were used at 6.4 pmol. Centri-Sep™ Columns (Princeton Separations, Inc., Adelphia, NJ) were used to purify the PCR products before sequencing on an Applied Biosystems 373A DNA Sequencer.

Prior to the sequencing described above, two sequencing reactions were completed using the T3 and T7 pBluescript SK^+ oligodeoxynucleotide primers (Stratagene). These reactions produced sequences for the first 350 nucleotides for the 5′-end of the first strand and the last 476 nucleotides for the 3′-end of the second strand. This and subsequent sequences were used in the preparation of ten synthetic 18-mer primers.

Analysis of the nucleotide sequence information was performed with the Intelligenetics Gene Works program (IntelliGenetics, Inc., Mountain View, CA). Searches of GenBank 85 (National Institute of Health, Bethesda, MD) were conducted using the IntelliGenetics Suite running on a VAX.

RESULTS

Probing With Homologous Vitellogenin Clones

Microvitellogenin cDNA from *M. sexta* hybridized under moderate stringency conditions to two restriction fragments (approximately 6 and 8 kb) of the EcoR1-digested DNA from *M. sexta* (Fig. 1A,B). This cDNA probe did not hybridize to distinct restriction fragments of the EcoR1-digested genomic DNA

from *S. frugiperda* (Fig. 1A) or from *L. dispar* (Fig. 1B) even when the blot was washed under moderate stringency conditions. The same Southern blot used in Figure 1A was reprobed with radiolabeled vitellogenin cDNA from *M. sexta*. Vitellogenin cDNA from *M. sexta* hybridized under moderate stringency conditions to two restriction fragments (approximately 7 and 8 kb) in the EcoR1-digested DNA from *M. sexta* (Fig. 1C). This cDNA probe did not hybridize to distinct restriction fragments of the EcoR1 digested genomic DNA from *S. frugiperda* or from *L. dispar* (Fig. 1C). The approximately 8 kb band hybridizing with the microvitellogenin (Fig. 1A,B) and vitellogenin (Fig. 1C) cDNAs disappeared under high stringency conditions (data not shown).

Microvitellogenin cDNA from *M. sexta* hybridized to an approximately 850 bp band in a Northern blot of *M. sexta* poly(A)$^+$RNA (Fig. 2A). A faint 850 bp band was also observed in *L. dispar* poly(A)$^+$RNA, but no bands were observed in the *S. frugiperda* poly(A)$^+$RNA even under low stringency conditions (Fig. 2A). The 850 bp band in *L. dispar* poly(A)$^+$RNA was more pronounced in a longer film exposure (4 days). In addition, other bands became apparent at about 1.5–2.5 and 3.0–5.0 kb in poly(A)$^+$RNA from *M. sexta, L. dispar,* and *S. frugiperda,* and at about 5.0 kb in poly(A)$^+$RNA from *S. frugiperda* (Fig. 2B). A band at 850 bp was not observed in *S. frugiperda* poly(A)$^+$RNA even after 4 days of autoradiography (Fig. 2B). The same Northern blot used in Figure 2 was reprobed with radiolabeled vitellogenin cDNA from *M. sexta*. Vitellogenin cDNA hybridized to an approximately 5.1 kb band in *M. sexta* poly(A)$^+$RNA (Fig. 3A), but no hybridization was not detected in the *L. dispar* and *S. frugiperda* poly(A)$^+$RNA samples even under low stringency conditions (Fig. 3A).

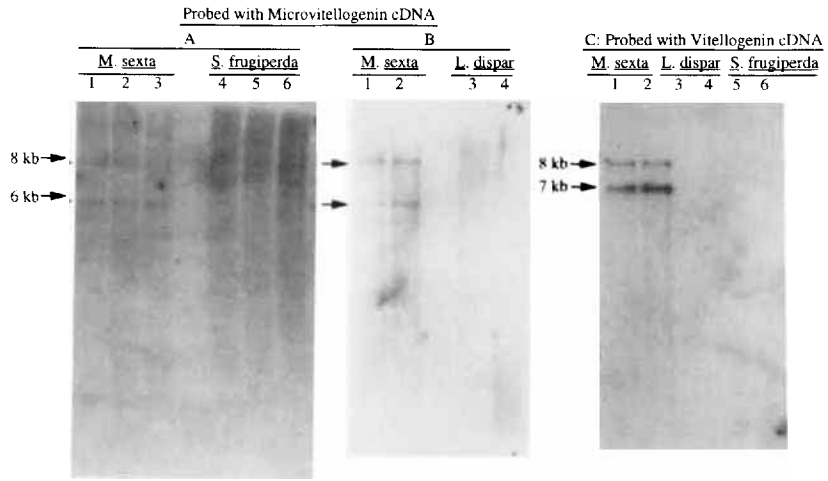

Fig. 1. Autoradiographs of Southern blots of EcoR1-digested genomic DNA probed with radiolabeled microvitellogenin (**A** and **B**) or vitellogenin **(C)** cDNA from *M. sexta*. The same Southern blot was used in A and C. Genomic DNA (about 10 μg) was digested for 12 h and separated on agarose electrophoresis run overnight. Each blot was placed in a bag containing phosphate hybridization buffer and radiolabeled probe. Hybridization was conducted for 12–16 h at 55°C. Moderate stringency conditions (two washes in phosphate buffer at 42°C) were used to remove excess unbound probe. Autoradiography was carried out for 5 days.

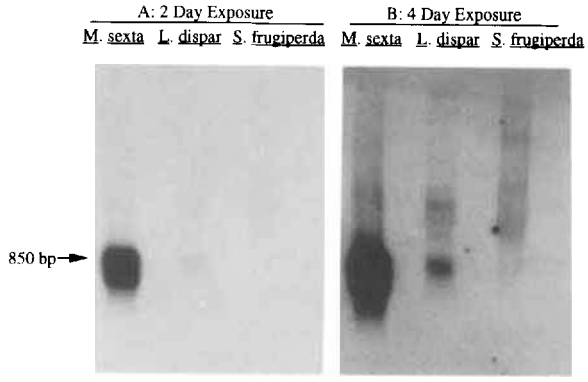

Fig. 2. Autoradiographs of a Northern blot probed with radiolabeled microvitellogenin cDNA from *M. sexta*. Insects compared were 1-day-old adults of female *M. sexta*, a 7-day-old last instar larvae of female *L. dispar*, and 2-day-old adults of female *S. frugiperda*. Autoradiography was carried out for 2 **(A)** and 4 days **(B)**. Approximately 10 μg of poly(A)$^+$RNA was loaded into each lane of a denaturing electrophoresis gel and electrophoresed for 3 h. The blot was incubated at 42°C for 2 h in hybridization buffer containing sheared and denatured herring sperm DNA. Hybridization with the radiolabeled probe was carried out at 42°C for 16 h. Low stringency conditions (two 5 min washes in 2 × SSPE containing 0.1% SDS, and a 5 min wash in 1 × SSPE containing 0.1% SDS) were used to remove excess unbound probe.

Clones pz64 and pz65 were the only female-specific cDNA clones that hybridized to a 5.3 kb band in *L. dispar* poly(A)$^+$RNA (Fig. 3B). These clones, whose inserts were both about 2.5 kb, did not hybridize to *M. sexta* or *S. frugiperda* poly(A)$^+$RNA even under low stringency conditions (Fig. 3B). Di-

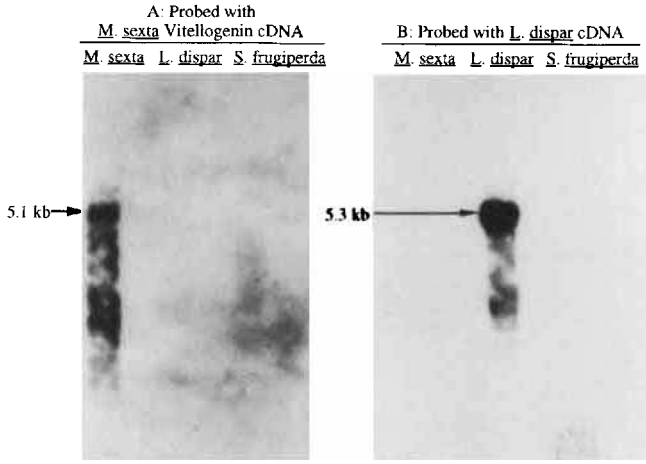

Fig. 3. Autoradiographs of the same Northern blot used in Figure 2 except that it was probed with radiolabeled vitellogenin cDNA from *M. sexta* **(A)** or the approximately 2.5 kb insert of cDNA clone pz64 from *L. dispar* **(B)**. Insects compared were 1-day-old adults of female *M. sexta*, 7-day-old last instar larvae of female *L. dispar*, and 2-day-old adults of female *S. frugiperda*. Electrophoresis, prehybridization, hybridization, and low stringency washing methods are the same as those for Figure 2. Autoradiography was carried out for 1 day.

gestion of pz64 and pz65 insert DNA with Hind III and Xba I produced the same banding pattern (data not shown). Therefore, only the cDNA insert in clone pz64 was used in further experiments.

Nucleotide Sequencing

The sequencing strategy and restriction map for cDNA clone pz64 are shown in Figure 4. A single large open reading frame was observed from position one of the clone to a termination codon at position 1870 (Fig. 5). This termination codon was followed by an AT-rich region with two putative polyadenylation signals, AATAAA, at positions 2069 and 2117. The deduced 623-residue amino acid sequence shows 38% identity with the carboxyl terminus of vitellogenin from *B. mori* (Yano et al., 1994) and 20% identity to the corresponding terminus of vitellogenin from *A. grandis* (Trewitt et al., 1992) (Fig. 6). *B. mori* and *A. grandis* have 17% identity with one another in the corresponding region. Alignment of the nucleotide sequence for *B. mori* and *L. dispar* vitellogenin cDNAs yields an overall sequence identity of 55% for the protein coding region shown in Figure 6 (alignment not shown). These sequence comparisons suggest that cDNA clone pz64 represents a partial transcript of *L. dispar* vitellogenin mRNA.

Female-Specificity and Developmental Timing of Vitellogenin Transcription in *L. dispar*

The pz64 cDNA from *L. dispar* strongly hybridized with a band at about 5.3 kb in the lanes containing poly(A)$^+$RNA from 7-day-old last instar female larvae (Fig. 7A). In a 3-day autoradiograph, a band of about 5.3 kb was also detected in poly(A)$^+$RNA from 2-day-old adult females (Fig. 7B). No hybridization was observed in 5-day-old last instar male larvae, 2-day-old last instar female larvae, or 3-day-old female pupae (Fig. 7B). A transcript near the size expected for microvitellogenin was not detected by any of the seven female-specific cDNA clones from *L. dispar* when tested with the same Northern blot shown in Figure 7 (data not shown).

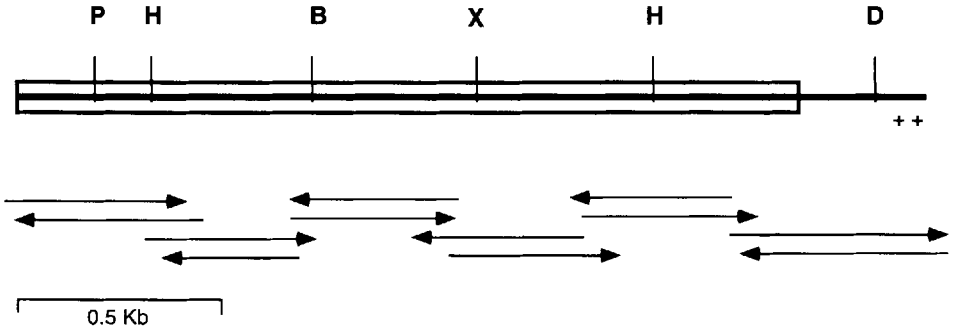

Fig. 4. Restriction map and sequencing strategy for cDNA clone pz64. Restriction sites are P (PvuII), H (HindIII), B (BclI), X (XbaI), and D (DraI). The open box denotes the open reading frame encoding 623 amino acids at the C-terminus of vitellogenin. Arrows indicate sequence obtained from internal primers or from the flanking T3 and T7 primers in the vector. Potential polyadenylation signals are denoted by +.

```
CTAAATTCCA CGGGCAAGTT CGGCGAAGCA CATAGATTTA GTGATGTTAC GCCCAATAGT GCACCTCGTC GCGAAGAAAT TGTCAAGCGT      90
L  N  S  T  G  K  F  G  E  A  H  R  F  S  D  V  T  P  N  S  A  P  R  R  E  E  I  V  K  R

GTTTCATCTG GTATTAAAAC TGCTACAGCG CGAGTTATAG ACTTCAGTGC ATCTTTTGAA GGCCTACAAA AACTAGAATA CGCAGTCACA     180
V  S  S  G  I  K  T  A  T  A  R  V  I  D  F  S  A  S  F  E  G  L  Q  K  L  E  Y  A  V  T

GCTGCGGTTG CAGGGAGCAT GGTTGATCTG AAAACCCAAT TTGCTGTTTT CATGGGGAGT CAGTCCGACA ACGGACAGAT CAATGCTGTC     270
A  A  V  A  G  S  M  V  D  L  K  T  Q  F  A  V  F  M  G  S  Q  S  D  N  G  Q  I  N  A  V

TTTAAGTTAC AAAAGCCTCA AATGGCACCA TTAGATTTCC ATAAAGCTTT GAATAGCGCA GTTAAATATC TTATCGAGGC CGATGTCACT     360
F  K  L  Q  K  P  Q  M  A  P  L  D  F  H  K  A  L  N  S  A  V  K  Y  L  I  E  A  D  V  T

TATGGAGAAA ACAGTAACAT TAACTTTAAG GGTCATACTG AACGTAGCCA GGAGTACGCG GAACAGTTAA AGAATAGTCT TTGGGCAAAC     450
Y  G  E  N  S  N  I  N  F  K  G  H  T  E  R  S  Q  E  Y  A  E  Q  L  K  N  S  L  W  A  N

CAGTGCGCTC AAGAGAATGC CCAGCAGAAC AAGTTCCAAC TAGGCTGCCA CAATGTGCTT ATAGAATCTC ATGCCCCAGA CCGCTTTAAG     540
Q  C  A  Q  E  N  A  Q  Q  N  K  F  Q  L  G  C  H  N  V  L  I  E  S  H  A  P  D  R  F  K

GCATCCATAA CATACAAACA TATACCCGCT GCACATACGG CTCTACTTGA TTCCTATATT CAGGGTTTAT GGAGTAAAGG CTTTGAATAT     630
A  S  I  T  Y  K  H  I  P  A  A  H  T  A  L  L  D  S  Y  I  Q  G  L  W  S  K  G  F  E  Y

AATCCATCAA AGAGGCTCCC TGTAGGTCAA ATAGAATTAG AGGCAAACGC TTCGTATGTT GATCAGACCG CAAATGTTGC GTGGACCTGG     720
N  P  S  K  R  L  P  V  G  Q  I  E  L  E  A  N  A  S  Y  V  D  Q  T  A  N  V  A  W  T  W

TGGAATGGAC AAGTACGGTT TAATAACTTA CCAAATACTT ATATTACACC CGCCTTAACG ACTGCCTACC AGCCTATTGG AATAGAAGAC     810
W  N  G  Q  V  R  F  N  M  L  P  N  T  Y  I  T  P  A  L  T  T  A  Y  Q  P  I  G  I  E  D

AGTTGGACTC ACTTTGCTAA CTCTTATTCA TATCATCAGT ATGAACCATT CTGCACCGTG GATGGCACAA AAGTGAAGAC ATTTAGTAAC     900
S  W  T  H  F  A  M  S  Y  S  Y  H  Q  Y  E  P  F  C  T  V  D  G  T  K  V  K  T  F  S  N

CGCGACTACA ACGTCACATT GCCCGAAATA TGGACTGTAT TAATGCACGC GCAGACAAAT TGGGAAGAAT TGGTCGTTCT TGCCAAGAGG     990
R  D  Y  N  V  T  L  P  E  I  W  T  V  L  M  H  A  Q  T  N  W  E  E  L  V  V  L  A  K  R

CCAAATGAGG CGAAGGCGAA GAGAGAAATT GGAAAAATAG GAAAATTGGA CTTATATATA TCACACAAGA CTGCGACGGG CAAACATCTA    1080
P  N  E  A  K  A  K  R  E  I  G  K  I  G  K  L  D  L  Y  I  S  H  K  T  A  T  G  K  H  L

GAAGTAAACA TTCCATATTC CGCTGCAAAT AATAAGGCTA ACGTAAAGGT GGAGACAAAC GCACAATTAG TCGCTGACGG TGACCTCACC    1170
E  V  N  I  P  Y  S  A  A  N  N  K  A  M  V  K  V  E  T  N  A  Q  L  V  A  D  G  D  L  T

ACTTATTGGG ACGATGTCGC AGAAACACCC CTCTTACAAT ATTCTAATCA TCCAGATCGT GTCTTGCTGC TCCATTTAAG TGATGGACTT    1260
T  Y  W  D  D  V  A  E  T  P  L  L  Q  Y  S  N  H  P  D  R  V  L  L  L  H  L  S  D  G  L

CACTTATTGT TTGATGGAAA ACGAGGCATC TTCACAACTT CTCAATACCG TAATATTACA AGAGGTATTT GCGGCCAGAA CAGTGGCGAT    1350
H  L  L  F  D  G  K  R  G  I  F  T  T  S  Q  Y  R  N  I  T  R  G  I  C  G  Q  N  S  G  D

CCACTAGACG ACTACAAGAC TCCATTAGGA ATAGTGGATC ACAGTCAACA TTTTGGAGCT GCCTTCACTT TAGACCTTGA GAAGACTAAT    1440
P  L  D  D  Y  K  T  P  L  G  I  V  D  H  S  Q  H  F  G  A  A  F  T  L  D  L  E  K  T  N

AGTCAGATAC AACAATGGAA GAAAATAGCT CAGGAGACTG CTTATCAACC CAAGCTTACG CACACTGTAA TCCTTCGTTT TGATGAAGAA    1530
S  Q  I  Q  Q  W  K  K  I  A  Q  E  T  A  Y  Q  P  K  L  T  H  T  V  I  L  R  F  D  E  E

TGGAAAATAG CTGGTGAACA AAAAGGGCTA GAATGGGGTT CTCAAAAAGT ATATAGGTCT AGGAGCTACC AGAAACAACG CGGTCCATGT    1620
W  K  I  A  G  E  Q  K  G  L  E  W  G  S  Q  K  V  Y  R  S  R  S  Y  Q  K  Q  R  G  P  C

CAAGTACAAA ATCAAGTGCA GTATCATGAA AATCATGGAG AGATATGCAT TACTACCACA CCCATATCTG CTTGCCAATC TCACTGTCAC    1710
Q  V  Q  N  Q  V  Q  Y  H  E  N  H  G  E  I  C  I  T  T  T  P  I  S  A  C  Q  S  H  C  H

AGCAGCAACT ATCAAGTACA AGCTGTTCAA GCGGTCTGCA AAGGCAAGAA AGATCCAGAG TTCAGAATGT ACAAGGACCA AATCCATCAA    1800
S  S  N  Y  Q  V  Q  A  V  Q  A  V  C  K  G  K  K  D  P  E  F  R  M  Y  K  D  Q  I  H  Q

GGTCAAAACC CTCAAGTAAC TGGAGTACCC AAGGTAGAGC AATATAGAGT ACCAACTACT TGCACAGAGT AAGAAAGTTG AGAAGGAAAA    1890
G  Q  N  P  Q  V  T  G  V  P  K  V  E  Q  Y  R  V  P  T  T  C  T  E  *

GAAAACGTGC ATAAGGACTA CGGTTTGTAC ATTTATGCGA ATATATATCA ATTTGGCTGT ATTTATGTAT TTAGTTCAAA TTTTAATTCA    1980

TATATTATGT GTAAATATAG TATTAAATGT ATTTAAATAT TATAGTACAT TTATATATTT ATAAAATATT GTATTATTAT GTAATTAGAA    2070

TAAAATATTA ATCAAATTAT GAAAACAAAA ATGAAATGTT ACTTATAATA AAATTATAAA AT                                    2132
```

Fig. 5. Nucleotide sequence of clone pz64, and deduced amino acid sequence for the large open reading frame. The stop codon is indicated by an asterisk. The two potential polyadenylation sites are underlined. This sequence has been deposited in Genbank with the accession number of U24161.

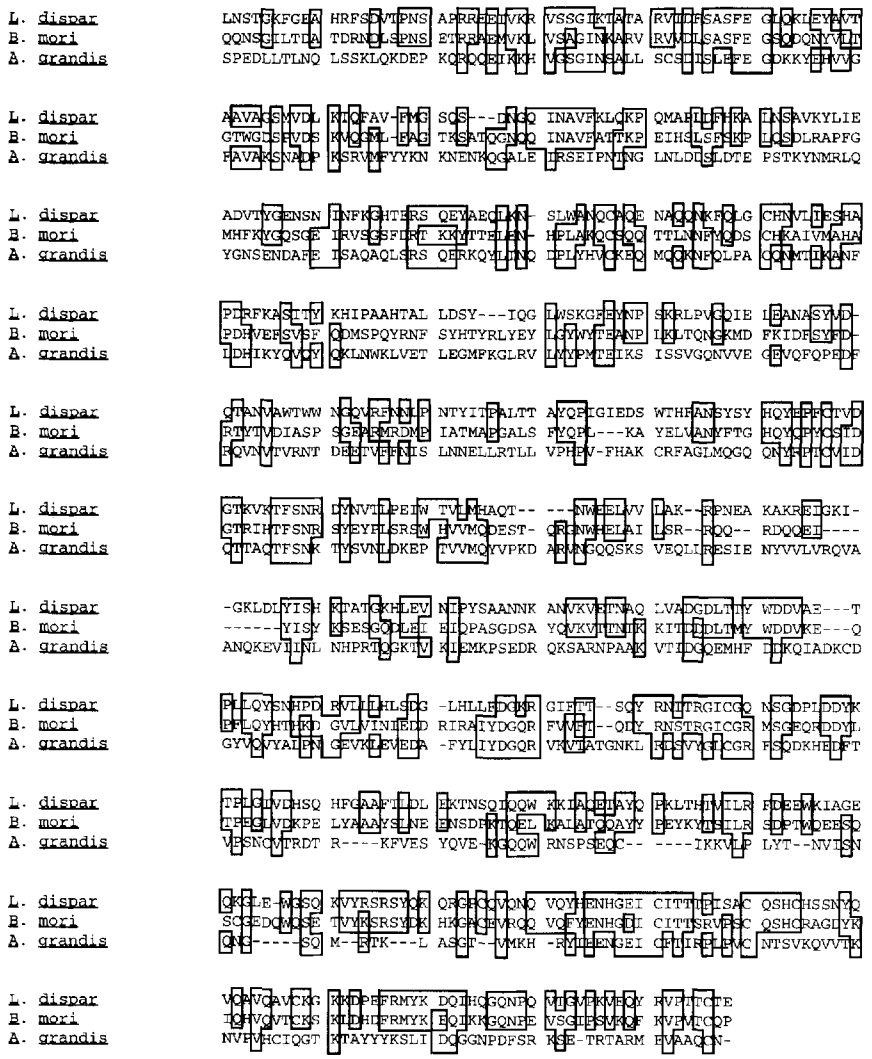

Fig. 6. Alignment of the 623 residue peptide sequence encoded by the large open reading frame of pz64 from *L. dispar*, the last 622 residues of *B. mori* vitellogenin (residues 1161–1782 of the precursor protein, Yano et al., 1994), and the last 618 residues of *A. grandis* vitellogenin (residues 1173–1790 of the precursor protein, Trewitt et al., 1992). Identical residues are boxed.

The pz64 cDNA probe hybridized to an approximately 5.3 kb transcript in poly(A) $^{+}$RNA from 5- and 7-day-old last instar female larvae (Fig. 8A). A low intensity band at about 5.3 kb was also observed in poly(A) $^{+}$RNA from 1- and 2-day-old female pupae, but not in 3- and 5-day-old female pupae (Fig. 8A). This low intensity band at about 5.3 kb reappears in 7-, 9-, and 11-day-old female pupae (Fig. 8A and B). In a 2.5 h autoradiograph of the Northern hybridization shown in Figure 8B, a low intensity band at about 5.3 kb is present in 1- and 3-day-old females (Fig. 8C).

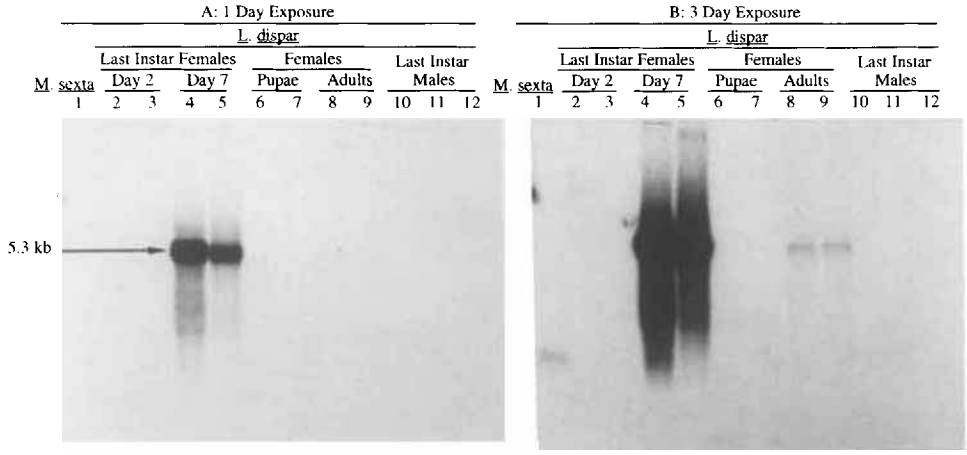

Fig. 7. Autoradiographs of a Northern analysis of poly(A)$^+$RNA from male and female *L. dispar* of various developmental periods. The ages for insects not listed in the figure are 1-day-old adults of female *M. sexta*, 3-day-old female *L. dispar* pupae, 1–3-day-old adults of female *L. dispar*, and 5-day-old last instar larvae of male *L. dispar*. The Northern blots were probed with the radiolabeled 2.5 kb insert of cDNA clone pz64 from *L. dispar*. Autoradiography was carried out for 1 **(A)** and 3 days **(B)**. Each lane contains 10 μg of poly(A)$^+$RNA. Electrophoresis, prehybridization, and hybridization methods are the same as those for Figure 2. Moderate stringency conditions (two 5 min washes in 2 × SSPE containing 0.1% SDS, a 5 min wash in 1 × SSPE containing 0.1% SDS, and a 5 min wash in 0.2 × SSPE containing 0.1% SDS) were used to remove excess unbound probe. Adults were not mated.

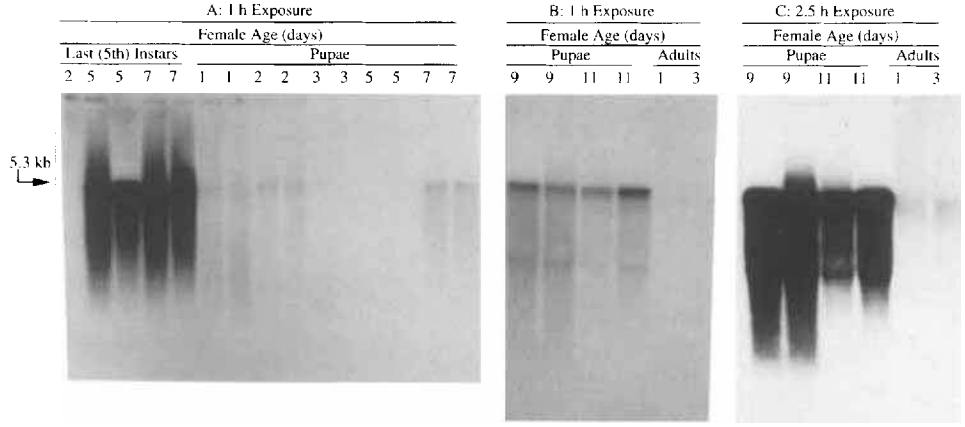

Fig. 8. Autoradiographs of Northern analyses of poly(A)$^+$RNA from female *L. dispar* of various developmental periods. The Northern blot was probed with the radiolabeled 2.5 kb insert of cDNA clone pz64 from *L. dispar*. Autoradiography was carried out for 1 **(A** and **B)** and 2.5 h **(C)**. Autoradiograph B and C are of the same Northern analysis. Each lane contains 10 μg of poly(A)$^+$RNA. Electrophoresis, prehybridization, and hybridization methods are the same as those for Figure 2. Moderate stringency washing conditions are the same as those for Figure 7. Autoradiography was carried out for 2.5 h.

Influence of Methoprene Treatment on Vitellogenin Transcription in Last Instar Larvae of *L. dispar*

The pz64 cDNA probe only hybridized to an approximately 5.3 kb transcript in poly(A)$^+$RNA from 5-day-old last instar larvae treated on day 2 with ethanol or with the lowest dose (10 nmol) of methoprene tested (Fig. 9). No hybridization of this probe could be detected in poly(A)$^+$RNA from larvae treated with larger doses of methoprene (Fig. 9).

DISCUSSION

Southern blot analyses under high stringency conditions indicate that the genes for microvitellogenin and vitellogenin in *M. sexta* are distinct and may each be present as a single copy in the genome of *M. sexta*. The presence of only a single copy of the vitellogenin gene has been observed in other insects (Barnett et al., 1980; Bradfield and Wyatt, 1983; Locke et al., 1987; Hiremath et al., 1994). Southern blot analysis under moderate stringency conditions (Fig. 1) suggests that there is another fragment (about 8 kb) in the genome of *M. sexta* having limited homology to both microvitellogenin and vitellogenin cDNAs. Homology between this fragment and the 6 and 7 kb genomic fragments hybridizing with the *M. sexta* microvitellogenin and vitellogenin cDNAs, respectively, is estimated to be no more than 77–85% (Bonner et al., 1973; for each 1% difference in nucleotide sequence there is a 1–1.5°C drop in the melting temperature). The lack of hybridization between microvitellogenin and vitellogenin cDNAs from *M. sexta* and homologous EcoR1 fragments in *S. frugiperda* or *L. dispar* suggests that genes for the vitellogenins in these latter species have much less than 77–85% homology with the *M. sexta* sequences. Nucleotide sequence similarity between the pz64 cDNA clone from

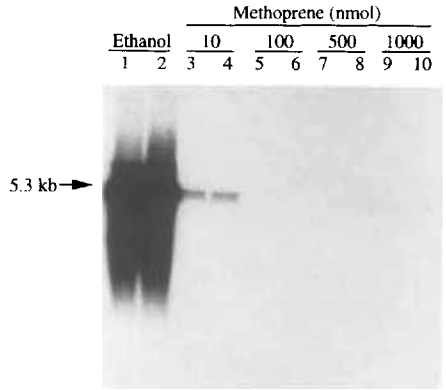

Fig. 9. Autoradiograph of a Northern analyses of poly(A)$^+$RNA from 5-day-old last instar larvae of female *L. dispar* that were topically treated on day 2 with ethanol or doses of the juvenile hormone analog methoprene. The Northern blot was probed with the radiolabeled 2.5 kb insert of cDNA clone pz64 from *L. dispar*. Each lane contains 10 μg of poly(A)$^+$RNA. Electrophoresis, prehybridizatioon, and hybridization methods are the same as those for Figure 2. Moderate stringency washing conditions are the same as those for Figure 7. Autoradiography was carried out for 2.5 h.

L. dispar and the corresponding portion of the *B. mori* vitellogenin gene is only 55%. Therefore, if *M. sexta* is as different from *L. dispar* as *B. mori* is, then hybridization to a heterologous probe would not be expected under the moderate stringency conditions used (Bonner et al., 1973).

For highly abundant transcripts like microvitellogenin and vitellogenin, detection of a homologous sequence by hybridization to a heterologous probe might be easier by Northern analysis than by Southern analysis. Microvitellogenin and vitellogenin cDNAs from *M. sexta* hybridized to bands in poly(A)$^+$RNA from adult female *M. sexta* that are the same sizes previously reported for microvitellogenin (about 850 bp; Wang et al., 1988) and vitellogenin (about 5.1 kb; Satyanaryana et al., 1994) mRNAs in *M. sexta*. These bands were detected with relatively short exposure times of 3–5 h (data not shown), indicating that microvitellogenin and vitellogenin cDNAs bind strongly as expected to their respective bands in *M. sexta* poly(A)$^+$RNA. In contrast, microvitellogenin and vitellogenin cDNAs from *M. sexta* hybridized only weakly or not at all to poly(A)$^+$RNA from last instar larvae of female *L. dispar* or from adults of female *S. frugiperda*, even when low stringency conditions were used (Figs. 2A,B and 3A,B). Transcripts present at 0.01% of the mRNA population should be detectable with an overnight exposure when 10 μg of poly(A)$^+$RNA is transferred to a Northern blot (Brown, 1988). Hiremath and Jones (1992) found the vitellogenin transcript in *L. dispar* to be highly abundant. They used ethidium bromide staining to easily detect the vitellogenin transcript in a denatured agarose gel containing 9 μg of poly(A)$^+$RNA from female *L. dispar* fat body. Apoproteins for high molecular weight vitellogenin in female *S. frugiperda* are abundant in the hemolymph of 2-day-old adults and are first detected on the day before adult eclosion (Fescemyer, unreported data). Yet Northern analysis of poly(A)$^+$RNA from female prepupae and 1-day-old adult *S. frugiperda* did not reveal hybridization of either microvitellogenin or vitellogenin cDNA from *M. sexta* to transcripts similar in size to transcripts for *M. sexta* microvitellogenin (Wang et al., 1988) or vitellogenin (Satyanarayana et al., 1994) (data not shown). The little or no hybridization of these *M. sexta* cDNAs to poly(A)$^+$RNA from female *L. dispar* and *S. frugiperda* in Northern analysis supports the conclusion that the similarity between the nucleotide sequences for microvitellogenin and vitellogenin in the three species is too low to be detected by hybridization.

The amount of sequence and structural homology among the lepidopteran vitellogenins is uncertain. A polyclonal antibody against *P. interpunctella* vitellogenin does not cross-react with that of *Galleria mellonella* (L.) even though they are both pyralids (Shirk, 1987). In addition, this antibody does not cross-react with vitellogenin from two other species of *Plodia* (Shirk, personal communication). On the other hand, a polyclonal antibody against vitellogenin from *M. sexta* does cross-react with vitellogenin from *H. cecropia* even though these species belong to different families (Osir et al., 1986). More molecular genetic research is needed to determine why particular epitopes of vitellogenin may be conserved among some Lepidoptera.

The 1.5–2.5 and 3.0–5.0 kb bands in Northerns probed with microvitellogenin cDNA from *M. sexta* (Fig. 2) were due to nonspecific hybridization of microvitellogenin cDNA to abundant mRNAs in *L. dispar* and *S.*

frugiperda and/or hybridization from some sequence similarity to *M. sexta* microvitellogenin. Further work with a cDNA probe synthesized from 18S rRNA (data not shown) revealed that hybridization of the microvitellogenin cDNA from *M. sexta* to the 1.5–2.5 bp bands was due to nonspecific hybridization to the 18S unit of rRNA that often contaminates poly(A)$^+$mRNA preparations (Fujiwara and Yamashita, 1992; Hiremath and Jones, 1992; Satyanarayana et al., 1994).

The similar approximately 2.5 kb inserts of *L. dispar* cDNA clones pz64 and pz65 hybridized to an approximately 5.3 kb transcript in poly(A)$^+$RNA from last instar larvae of female *L. dispar*. This transcript is similar in size to the vitellogenin transcript in *M. sexta* (Fig. 3) and to the vitellogenin mRNA isolated from last instar larvae of female *L. dispar* as reported by Hiremath and Jones (1992). However, these clones did not hybridize to poly(A)$^+$RNA from 1-day-old adults of female *M. sexta* or from 2-day-old adults of female *S. frugiperda*. This finding provides further support for the conclusion that similarity between the nucleotide sequences for vitellogenin in these three species is too low to be detected by hybridization.

The deduced amino acid sequence of the pz64 insert from *L. dispar* had no recognizable similarity with the sequences for *M. sexta* microvitellogenin or the low molecular weight vitellogenins and yolk proteins of *D. melanogaster* (Bownes et al., 1988) and *C. capitata* (Blumenthal and Zucker-Aprison, 1987; Rina and Savakis, 1991) (data not shown). The alignment of the deduced amino acid sequence of pz64 with the sequences for higher molecular weight vitellogenin from *A. grandis* (Trewitt et al., 1992) and *B. mori* (Yano et al., 1994) indicates that the pz64 insert is a partial cDNA coding for the 3′-end of a high molecular weight vitellogenin. These alignments therefore indicate that the *L. dispar* vitellogenin gene is a member of the nematode-vertebrate-insect family of yolk protein genes for high molecular weight vitellogenins (Nardelli et al., 1987; Trewitt et al., 1992).

The approximately 5.3 kb vitellogenin transcript hybridizing with the pz64 insert is expressed only in female *L. dispar* (Fig. 7). This finding is supported by other investigations that found vitellogenin expression (Hiremath and Jones, 1992; Hiremath and Eshita, 1992), vitellogenin synthesis (Fescemyer et al., 1992), and the presence of vitellogenin apoproteins in the hemolymph (Davis et al., 1990a; Fescemyer et al., 1992; Hiremath and Jones, 1992; Kelly et al., 1992) to be female-specific in *L. dispar*. Presence of the 5.3 kb vitellogenin transcript in poly(A)$^+$RNA was also found to vary with the development of *L. dispar* females (Fig. 8). This transcript was not detected in 2-day-old last instar larvae, although an enzyme-linked immunoabsorbant assay (ELISA) had detected trace amounts of vitellogenin (6 μg/ml) in the hemolymph of 2-day-old last instar female larvae (Lamison et al., 1991). The 5.3 kb transcript was detected in 3-day-old last instar larvae (data not shown) and was found to be very abundant in 7-day-old last instar female larvae (Fig. 8). These findings are generally consistent with those in the literature. The ELISA detected only trace amounts of vitellogenin (6 μg/ml) in the hemolymph of 2-day-old last instar female larvae and no hemolymph vitellogenin in younger larvae (Lamison et al., 1991). Hiremath and Jones (1992) first detected the vitellogenin transcript in 6-day-old last instar larvae. Hemolymph vitellogenin

reached a maximum level of 8.25 mg/ml in 6-day-old last instar larvae (Lamison et al., 1991).

Transcription of vitellogenin DNA is greatly reduced in pupae of female *L. dispar* when compared to that in 7-day-old last instar larvae. The 5.3 kb vitellogenin transcript was present at a low level in poly(A)$^+$RNA from all ages of pupae tested except 3- and 5-day-old pupae (Fig. 8). This transcript was also detected in young adult females (Fig. 8). Hemolymph vitellogenin gradually declined in concentration during the pupal stage, but remained above 1 mg/ml (Lamison et al., 1991) even in young adults. Oogenesis in *L. dispar* occurs during the pupal stage, and adult females eclose with nearly a full complement of eggs (Davis et al., 1990a). Most of the vitellogenin needed for oogenesis is probably expressed during the last larval instar and only low levels of expression are needed to maintain oogenesis during the pupal and early adult stages. Regulation of vitellogenin transcription during the pupal stage probably differs from its regulation in last instar larvae. This regulatory mechanism in pupae could involve the second, prepupal peak of juvenile hormone, as in *M. sexta* (Satyanarayana et al., 1994), or 20-hydroxyecdysone whose hemolymph titer peaks on day 3 in female pupae (Schnee et al., 1984), or the decrease in hemolymph vitellogenin titer as a result of yolk uptake during vitellogenesis. Further studies need to address the role of these and possibly other factors in regulating vitellogenin expression and oogenesis during the pupal stage.

Synthesis of the approximately 5.3 kb vitellogenin transcript in early last instar larvae of female *L. dispar* appears to be repressed by the high titer of juvenile hormone (Tanaka et al., 1989) during the first 2 days of the last instar. This conclusion is supported by other investigations that found vitellogenin expression (Hiremath and Jones, 1992; Hiremath and Eshita, 1992; Fescemyer et al., 1992) and the presence of vitellogenin apoproteins in the hemolymph (Davis et al., 1990a; Fescemyer et al., 1992; Hiremath and Jones, 1992; Kelly et al., 1992) to be suppressed in last instar larvae by juvenile hormone or its analogs, methoprene and fenoxycarb. The female-specificity of the 5.3 kb vitellogenin transcript and its repression by juvenile hormone supports the conclusion that this transcript is vitellogenin mRNA. One of the primary functions of juvenile hormone is suppression of improper maturation in larvae. Information about how juvenile hormone suppresses vitellogenin expression in *L. dispar* will lead to a better understanding of how juvenile hormone represses the expression of genes involved in larval maturation and metamorphosis.

LITERATURE CITED

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (eds) (1988): Current Protocols in Molecular Biology, vol. 2. New York: John Wiley & Sons, pp 3.1–3.3.

Barnett T, Pachl C, Gergen JP, Wensink PC (1980): The isolation and characterization of *Drosophila* yolk protein genes. Cell 21:729–738.

Benton WD, Davis RW (1977): Screening λgt recombinant clones by hybridization to single plaques in situ. Science 196:180–182.

Blumenthal T, Zucker-Aprison E (1987): Evolution and regulation of vitellogenin genes. In O'Conner JD (ed): Molecular Biology of Invertebrate Development. New York: Alan R. Liss, pp 3–19.

Bonner TI, Brenner DJ, Neufeld BR, Britten RJ (1973): Reduction in the rate of DNA reassociation by sequence divergence. J Mol Biol 81:123–135.

Bownes M, Shirras A, Blair M, Collins J, Coulson A (1988): Evidence that insect embryogenesis is regulated by ecdysteroids released from yolk proteins. Proc Natl Acad Sci USA 85:1554–1557.

Bradfield JY, Wyatt GR (1983): X-linkage of a vitellogenin gene in *Locusta migratoria*. Chromosoma 88:190–193.

Brown T (1988): Hybridization analysis of DNA blots. In Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (eds): Current Protocols in Molecular Biology, vol. 1. New York: John Wiley & Sons, pp 2.10.1–2.10.16.

Chomczynski P (1992): One-hour downward alkaline capillary transfer for blotting of DNA and RNA. Anal Biochem 201:134–139.

Chomczynski P, Sacchi N (1987): Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159.

Cusson M, Yu CG, Carruthers K, Wyatt GR, Tobe SS, McNeil JN (1994): Regulation of vitellogenin production in armyworm moths, *Pseudaletia unipuncta*. J Insect Physiol 40:129–136.

Davis RE, Kelly TJ, Masler EP, Thyagaraja BS, Paramasivan V, Fescemyer HW, Bell RA, Borkovec AB (1990a): Vitellogenesis in the gypsy moth, *Lymantria dispar* (L.): Characterization of hemolymph vitellogenin, ovarian weight, follicle growth and vitellin content. Invert Reprod Dev 18:137–145.

Davis RE, Kelly TJ, Masler EP, Fescemyer HW, Thyagaraja BS, Borkovec AB (1990b): Hormonal control of vitellogenesis in the gypsy moth *Lymantria dispar* (L.): Suppression of haemolymph vitellogenin by the juvenile hormone analogue, methoprene. J Insect Physiol 36:231–238.

Engelmann F (1983): Vitellogenesis controlled by juvenile hormone. In Downer R.G.H., Laufer H. (eds): Endocrinology of Insects. New York: Alan R. Liss, pp 259–270.

Fescemyer HW, Masler EP, Davis RE, Kelly TJ (1992): Vitellogenin synthesis in female larvae of the gypsy moth, *Lymantria dispar* (L.): Suppression by juvenile hormone. Comp Biochem Physiol 103B:533–542.

Fujiwara Y, Yamashita O (1992): Gene structure of *Bombyx mori* larval serum protein (BmLSP). Insect Mol Biol 1:63–69.

Gerard GF (1988): Synthesis of high specific activity cDNA. Focus 10:12–13.

Hagedorn HH, Kunkel JG (1979): Vitellogenin and vitellin in insects. Annu Rev Entomol 24:475–505.

Hiremath S, Eshita S (1992): Purification and characterization of vitellogenin from the gypsy moth, *Lymantria dispar*. Insect Biochem Mol Biol 22:605–611.

Hiremath S, Jones D (1992): Juvenile hormone regulation of vitellogenin in the gypsy moth, *Lymantria dispar*: Suppression of vitellogenin mRNA in the fat body. J Insect Physiol 38:461–474.

Hiremath S, Lehtoma K, Nagarajan M (1994): cDNA cloning and expression of the large subunit of juvenile hormone-regulated vitellogenin from *Lymantria dispar.* J Insect Physiol 40:813–821.

Huynh TV, Young RA, Davis RW (1985): Constructing and screening cDNA libraries in λgt10 and λgt11. In Glover DM, Hames BD (eds): DNA Cloning: A Practical Approach, vol. 1. Oxford: IRL Press, pp 48–78.

Kawooya JK, Law JH (1983): Purification and properties of microvitellogenin of *Manduca sexta* role of juvenile hormone in appearance and uptake. Biochem Biophys Res Commun 117:643–650.

Kelly TJ, Davis RE, Fescemyer HW, Thyagaraja BS, Bell RA, Masler EP (1992): Regulation of gypsy moth vitellogenesis: A novel juvenile hormone suppression mechanism. In Mauchamp B, Couillaud F, Baehr JC (eds): Insect Juvenile Hormone Research: Fundamental and Applied Approaches—Chemistry, Biochemistry and Mode of Action. Paris: INRA Press, pp 173–181.

King EG, Hartley GG (1985): *Diatraea saccharalis.* In Singh P, Moore RF (eds): Handbook of Insect Rearing, vol. 2. Amsterdam: Elsevier, pp 265–270.

Kirby KS (1957): A new method for the isolation of deoxyribonucleic acids: Evidence on the nature of bonds between deoxyribonucleic acid and protein. Biochem J 66:495–504.

Kulakosky PC, Telfer WH (1989): Kinetics of yolk precursor uptake in *Hyalophora cecropia:* Stimulation of microvitellogenin endocytosis by vitellogenin. Insect Biochem 19:367–373.

Kunkel JG, Nordin JH (1985): Yolk Proteins. In Kerkut GA, Gilbert LI (eds): Comprehensive Insect Physiology, Biochemistry and Pharmacology, vol. 1. Oxford: Pergamon Press, pp 83–111.

Lamison CD, Ballarino J, Ma M (1991): Temporal events of gypsy moth vitellogenesis and ovarian development. Physiol Entomol 16:201–209.

Lehrach H, Diamond D, Wozney JM, Boedtker H (1977): RNA molecular weight determinations by gel electrophoresis under denaturing conditions: a critical reexamination. Biochemistry 16:4743–4751.

Locke J, White BN, Wyatt GR (1987): Cloning and 5′ end nucleotide sequences of two juvenile hormone-inductable vitellogenin genes of the African migratory locust. DNA 6:331–342.

Nardelli D, Gerber-Huber S, van het Schip FD, Gruber M, Ab G, Wahli W (1987): Vertebrate and nematode genes coding for yolk proteins are derived from a common ancestor. Biochemistry 26:6397–6402.

Osir EO, Wells MA, Law JH (1986): Studies on vitellogenin from the tobacco hornworm *Manduca sexta.* Arch Insect Biochem Physiol 3:217–233.

Pan ML, Wyatt GR (1976): Control of vitellogenin synthesis in the monarch butterfly by juvenile hormone. Dev Biol 54:127–134.

Pashley DP (1986): Host associated genetic differentiation in fall armyworm: A sibling species complex? Ann Entomol Soc Am 79:898–904.

Pashley DP (1989): Host-associated differentiation in armyworms (Lepidoptera: Noctuidae): An allozymic and mitochondrial DNA perspective. In Loxdale HD, den Hollander J (eds): Electrophoretic Studies on Agricultural Pests. Oxford: Clarendon Press, pp 103–114.

Perkins WD (1979): Laboratory rearing of the fall armyworm. Fla Entomol 62:87–91.

Puissant C, Houdebine L-M (1990): An improvement of the single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Biotechniques 8:148–149.

Rina M, Savakis C (1991): A cluster of vitellogenin genes in the Mediterranean fruit fly *Ceratitis capitata:* Sequence and structural conservation in dipteran yolk proteins and their genes. Genetics 127:769–780.

Satyanarayana K, Bhaskaran G, Dahm KH, Meola R (1992): Regulation of vitellogenin synthesis by juvenile hormone in the corn earworm, *Helicoverpa zea.* Invert Reprod Dev 21:169–178.

Satyanarayana K, Bradfield JY, Bhaskaran G, Dahm KH (1994): Stimulation of vitellogenin production by methoprene in prepupae and pupae of *Manduca sexta.* Arch Insect Biochem Physiol 25:21–37.

Schnee ME, Ma M, Kelly TJ (1984): Hormonal basis of adult eclosion in the gypsy moth (Lepidoptera: Lymantriidae). J Insect Physiol 30:351–356.

Shirk PD (1987): Comparison of yolk production in seven pyralid moth species. Int J Invert Reprod Dev 11:173–188.

Shirk PD, Zimowska G, Silhacek DL, Shaaya E (1992): Initiation of vitellogenesis in pharate adult females of the Indianmeal moth, *Plodia interpunctella.* Arch Insect Biochem Physiol 21:53–63.

Southern EM (1975): Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517.

Tanaka S, Chang MT, Denlinger DL, Abdel-Aal YAI (1989): Developmental landmarks and the activity of juvenile hormone and juvenile hormone esterase during the last stadium and pupa of *Lymantria dispar.* J Insect Physiol 35:897–905.

Tautz D, Renz M (1983): An optimized freeze squeeze method for the recovery of DNA from agarose gels. Anal Biochem 132:14–19.

Trewitt PM, Heilmann LJ, Degrugillier SS, Kumaran AK (1992): The boll weevil vitellogenin gene: Nucleotide sequence, structure, and evolutionary relationship to nematode and vertebrate vitellogenin genes. J Mol Evol 34:478–492.

Voytas D (1988): Resolution and recovery of large DNA fragments. In Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (eds): Current Protocols in Molecular Biology, vol. 1. New York: John Wiley & Sons, pp 2.5.1–2.5.9.

Wang X-Y, Cole KD, Law JH (1988): cDNA cloning and deduced amino acid sequence of microvitellogenin, a female specific hemolymph and egg protein from the tobacco hornworm, *Manduca sexta.* J Biol Chem 263:8851–8855.

Wang X-Y, Cole KD, Law JH (1989): The nucleotide sequence of a microvitellogenin encoding gene from the tobacco hornworm, *Manduca sexta.* Gene 80:259–268.

Yano K-I, Sakurai MT, Watabe S, Izumi S, Tomino S (1994): Structure and expression of mRNA for vitellogenin in *Bombyx mori.* Biochim Biophys Acta 1218:1–10.